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Trophic plasticity in the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) from the north central Gulf of Mexico

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Abstract Quantifying the trophic role of sharks in coastal ecosystems is crucial for the construction of accurate ecosystem models. This is particularly important for wide-ranging species like the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*), ubiquitous across the northern Gulf of Mexico. We used gut content and stable isotope analyses to determine if differences in abundance of Atlantic sharpnose sharks in the waters around Mobile Bay, Alabama translated into differences in dietary sources or trophic position among sharks sampled east and west relative to the mouth of the bay. Gut content analysis suggested that Atlantic sharpnose sharks eat primarily teleost fishes (%IRI>90% across size classes), and both stomach content and stable isotope analyses highlighted an ontogenetic shift in diet. Nitrogen stable isotope data from liver and muscle tissues indicated regional shifts in trophic position for Atlantic sharpnose sharks. The mixing model SIAR (stable isotope analysis in R) v.4.0.2 was used to suggest possible contributions from likely prey items for Atlantic sharpnose sharks sampled east and west of Mobile Bay. Portunid crabs and shrimp made higher contributions to the diet of Atlantic sharpnose sharks in the western region, compared to higher and more variable contributions from fish like croaker (*Micropogonias undulatus*) and hardhead catfish (*Arius felis*) in the eastern region. Our results suggest trophic plasticity in Atlantic sharpnose sharks, findings that emphasize the importance of examining regional variation in trophic position when constructing coastal foodweb models.

Keywords SIAR · Trophic ecology · Trophic plasticity · Forage base

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Introduction

The Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) is the most ubiquitous small coastal shark in the US Atlantic Ocean (Cortés 2002). Although aspects of the life history (Parsons 1983; Carlson and Baremore 2003), movement (Carlson et al. 2008), distribution (Carlson and Brusher 1999; Parsons and Hoffmayer 2005; Drymon et al. 2010) and feeding ecology (Hoffmayer and Parsons 2003; Bethea et al.

2004, 2006) of this shark have been documented in the Gulf of Mexico (GOM), little information exists on the role of this shark in the food web. A recent examination of catch series data for Atlantic sharpnose shark across the GOM revealed an east-west gradient; catch-per-unit-effort (CPUE) was higher west of Mobile Bay, Alabama compared to east of the bay, potentially driven by differences in primary productivity and forage fish biomass between the regions (Drymon 2010). Effective ecosystem based management requires that the trophic position of higher level consumers be characterized (Stevens et al. 2000). Traditionally, trophic position for most sharks has been viewed as static in foodweb models (Stevens et al. 2000; Kitchell et al. 2002); however, intraspecific variation in trophic level among higher order consumers has been shown to occur across relatively small spatial scales in the GOM (Rush et al. 2010).

Feeding habits and hence trophic position for sharks have historically been defined through analysis of stomach contents; however, several issues limit the utility of this approach (Hobson and Welch 1992). Although conceptually straightforward, stomach content analysis is limited by its 'snapshot' nature and inter/intraspecific variation in digestion rates. These problems often result in the failure of dietary analysis to fully predict trophic level (Polunin and Pinnegar 2002).

A complement to stomach content analysis is stable isotope analysis. This technique is reliant on the fact that lighter, more common isotopes of an element are preferentially respired or excreted during metabolism, leading to a relative accumulation of the heavier isotope in the tissue of the organism. Naturally occurring stable isotope ratios of carbon (^{13}C:^{12}C) and nitrogen (^{15}N:^{14}N) are particularly useful for discerning relationships between an organism and its prey (Peterson and Fry 1987). The carbon value of a consumer is similar to that of it's prey, thereby making $\delta^{13}C$ useful for determining the ultimate source of primary production at the base of a consumer's diet (DeNiro and Epstein 1978). Conversely, $\delta^{15}N$ values increase with increasing trophic level, making them useful indicators of trophic position (Peterson and Fry 1987). No studies have used both stable isotope and stomach content analyses to document feeding habits for the Atlantic sharpnose shark.

To determine if differences in forage fish biomass (Drymon 2010) are associated with changes in diet and ultimately trophic position for the Atlantic sharpnose shark, we conducted stable isotope analysis of C and N in muscle and liver tissues for this species. Since stable isotope turnover occurs more rapidly in elasmobranch liver tissue compared to muscle (MacNeil et al. 2006), sampling both tissues will provide insight into dietary habits at different temporal scales. Our goals were to 1) determine the trophic position (using $\delta^{15}N$) and contribution of benthic versus pelagic organic matter (using $\delta^{13}C$), 2) identify the extent to which $\delta^{15}N$ varied spatially (east or west of Mobile Bay) and temporally (throughout the year), and to 3) compare the feeding ecology of the Atlantic sharpnose shark using stomach content versus stable isotope analyses.

Materials and methods

Sample collection

A cooperative longline survey was initiated in May of 2006 by the National Oceanic and Atmospheric Administration (NOAA) Mississippi (MS) Labs and the Dauphin Island Sea Lab (DISL) to survey shark species assemblages in the coastal waters of Mississippi and Alabama. This survey was designed as a companion survey to the NOAA MS Labs' annual bottom-longline survey, meaning gear and protocols used in both surveys were identical to facilitate data comparisons. For a complete description of longline methods, see Driggers et al. (2008). Longline sampling was conducted from 2006–2008 on NOAA research vessels *R/V* HST, *R/V* Gandy and *R/V* Caretta. Based on initial data from May 2006–February 2007, longline sampling was not conducted during the winter months (December, January and February) due to the complete absence of sharks in our survey area (n=21 sets); therefore, only data from March 2007–November 2008 are used for this analysis.

This survey employed a random stratified block design, with four blocks established along the Mississippi/Alabama coast. Each block was 37 km east to west and extended from the shoreline to approximately the 20 m isobath. Blocks one and two were located west of 88° W longitude (Mobile Bay),

whereas blocks three and four were located east of 88° (Fig. 1a). Sampling was evenly allocated and replicated within each block along three depth strata: 0–5 m, 5–10 m and 10–20 m. Twelve stations were selected at random each month: six in one of the eastern blocks (blocks 3 or 4), and six in one of the western blocks (blocks 1 or 2). This survey design ensured equal station dispersion within the block (two stations across each depth stratum), while always sampling one eastern and one western block each month. At each station, a single bottom-longline was set and soaked for 1 h. The main line consisted of 1.85 km (1 nm) of 4 mm monofilament (545 kg test) sampled with 100 gangions; each gangion was made of 3.66 m of 3 mm (320 kg test) monofilament. Gangions consisted of a longline snap and a 15/0 circle hook, baited with Atlantic mackerel (*Scomber scombrus*).

All sharks captured were enumerated by species and morphological measurements collected. Straight line length, weight and maturity data were collected from sharks that could be safely boated, removed from the mainline, unhooked and identified to species. All length measurements originated at the tip of the rostrum and terminated at the origin of the precaudal pit, the noticeable fork in the tail, and the upper lobe of the caudal fin in a stretched position for precaudal (PCL), fork (FL), and total lengths (TL), respectively. Maturity in males was assessed following Clark and von Schmidt (1965). Most sharks were tagged and released.

Atlantic sharpnose sharks were sacrificed to collect tissue for stable isotope analysis. A section of white muscle below the primary dorsal fin (Estrada et al. 2003; MacNeil et al. 2005), a portion of the left lobe of the liver (Fisk et al. 2002; MacNeil et al. 2006) and the entire stomach were removed from all Atlantic sharpnose sharks sacrificed. All samples were placed on ice or frozen shipboard in a −20°C freezer awaiting laboratory analysis.

To characterize the prey base available to Atlantic sharpnose sharks, data from the Alabama Marine Resources Division (ALMRD) Fisheries Assessment and Monitoring Program (FAMP) were compiled for all demersal species captured by this trawl survey during the period of our analysis (2007–2008). The ALMRD trawl survey sampled multiple inshore stations throughout coastal Alabama each month. For our analysis, two of these stations were chosen based on their location relative to our survey design; one station in the western region (88° 03.3′ W) and one station in the eastern region (87° 33.7′) (Fig. 1a).

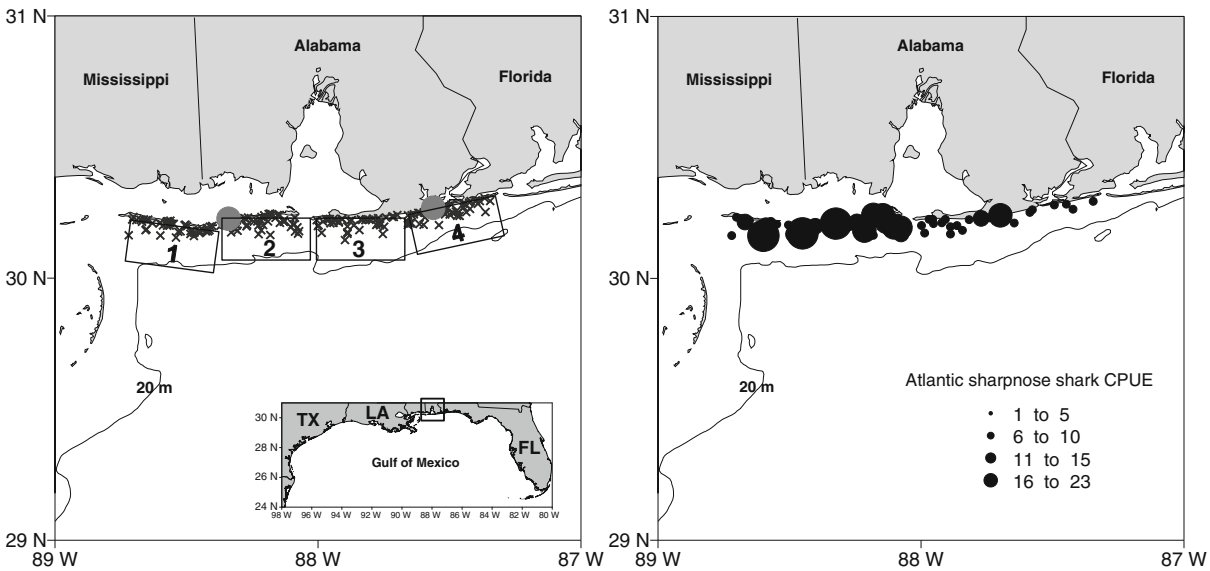

Fig. 1 **a** Survey area and sampling design. Black x's indicate the location of each longline set, and grey circles indicate locations of ALMRD FAMP monthly trawl surveys used in this analysis. Boxes represent western (1 and 2) and eastern (3 and 4) regions **b** Catch per unit effort (CPUE, sharks/100 hooks/hour, black circles) for Atlantic sharpnose sharks sampled during March–November, 2007 and 2008

Trawl samples were collected with a 5 m otter trawl fitted with 40×60 cm wooden doors. The net was constructed of 35 mm stretched mesh with a 45 mm cod-end fitted with a 4.7 mm liner.

Data from the ALMRD FAMP survey were used both to characterize differences in potential prey biomass between regions, and to calculate trophic position of potential prey species. Total biomass for fish and invertebrates was calculated for the years 2007 and 2008. Data from this survey comprised 157 species; however, 29 of these species (Cnidarians, gastropods and bivalves) were deemed unrealistic as dietary items for Atlantic sharpnose shark and were removed from the dataset. Total biomass for the remaining fish and invertebrates (n=128) was calculated for the years 2007 and 2008 by season (spring, summer and fall) and region (western and eastern). We chose to examine overall biomass of these two prey types (fish and invertebrates) rather than specific species to examine whether Atlantic sharpnose sharks were responding to gradients in available biomass. In addition, we used muscle samples taken from a subset of these potential prey species (n=101 individuals, 7 species) for which sufficient tissue had been collected during 2008 to assess trophic position and C source of potential prey. Because of low sample size, potential prey species were pooled across spring, summer and fall. ALMRD trawl surveys do not routinely capture all fish common to the diet of Atlantic sharpnose sharks (Hoffmayer and Parsons 2003); for this reason, isotope data (*Anchoa* sp., n=11, RHC unpubl. data) from fish sampled in the same area were added to our analysis.

Stable isotope analysis

To identify trophic position and C source we measured stable isotope ratios of N and C in white muscle and liver tissue from Atlantic sharpnose sharks and muscle tissue from potential prey. Tissues were rinsed in deionized water, subsampled (0.5–1.0 g), freeze dried for 48 h in a Labconco lypholizer, ground into powder with a mortar and pestle and packed into 2 mg (±0.05) aliquots in tin capsules (Elementar Americas) for stable isotope analysis. Prior to subsampling, a modified Folch et al. (1957) lipid extraction (Post et al. 2007) was performed on shark liver tissue to remove lipids, as lipids can lead to a depletion in ^{13}C (Park and Epstein 1961). C:N ratios in shark liver tissue and potential prey muscle were used as relative measures of lipid content (Post et al. 2007). C:N ratios in potential prey items were all less than 3.5. All shark tissues with C:N values greater than 3.5 were lipid extracted. Following lipid extraction, tissues were placed in a drying oven at 60°C for 48 h, or until a constant weight was reached. Examination of post lipid-extracted C:N ratios indicated that lipids were incompletely removed from liver tissue; therefore, a tissue-specific mathematical normalization was conducted such that $\delta^{13}C'$-$\delta^{13}C = -2.976+3.093[\ln(C:N)]$, where $\delta^{13}C'$ = the lipid normalized value (Logan et al. 2008). Stable isotope ratios of ^{13}C:^{12}C and ^{15}N:^{14}N were determined using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of California Davis Stable Isotope Facility. Stable isotope ratios were expressed in delta notation per the following formula: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is the heavy isotope, R_{sample} is the ratio of heavy to light isotope in the sample, and $R_{standard}$ is the ratio of heavy to light isotope in the reference standard. During analysis, several replicates of at least two laboratory standards (calibrated against NIST reference IAEA-N1, IAEA-N2, IAEA-N3, USGS-40 and USGS-41) were included (SD=0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$). The standard reference material was Pee Dee Belemnite for C and atmospheric N_2 for N.

Trophic position (TP) for Atlantic sharpnose sharks was estimated following Post (2002): $TP = \lambda + (\delta^{15}N_c - \delta^{15}N_{base})/\Delta_n$, where λ is the trophic level of the base, $\delta^{15}N_c$ and $\delta^{15}N_{base}$ are the nitrogen isotope values of the consumer and base, respectively, and Δ_n is the trophic fractionation factor for nitrogen. We set λ=2 (a primary consumer) to reduce the propagation of error associated with the variability in Δ_n (Vander Zanden and Rassmussen 2001; Wolf et al. 2009). We used $\delta^{15}N$ values chosen from primary consumers (polycheates and bivalves) that are as close as possible to true herbivores, and for which values were collected within our study area (RHC unpubl. data). We chose a taxon and tissue specific Δ_n value of 2.28 for bulk fish tissue following the experimentally determined trophic fractionation factors for shark tissues (Hussey et al. 2010).

The relative contribution from different prey groups to the isotope signature of sharpnose sharks sampled in the eastern and western regions was

estimated using the isotope mixing model SIAR (stable isotope analysis in R) version 4.0.2 (Parnell et al. 2010) in the R statistical platform (CRAN 2009). This model uses a Bayesian framework to estimate the proportional contribution of prey to a consumer diet, while including variability in model inputs such as trophic fractionation values. Mean $\delta^{13}C$ and $\delta^{15}N$ values (±SD) for prey species were included as sources. Following Hussey et al. (2010), tissue specific trophic fractionation values of 2.28‰ and 1.13‰ (for $\Delta^{15}N$ and $\Delta^{13}C$, respectively) were used as inputs, combined with a standard deviation value of 1 for both $\delta^{13}C$ and $\delta^{15}N$. This relatively large error term was chosen to encompass the variability shown in recent meta-analyses (McCutchan et al. 2003, Sweeting et al. 2007, Caut et al. 2009), following Inger et al. (2010). C:N ratios for potential prey were used to calculate elemental concentrations, which were also added to the mixing model.

Stomach content analysis

Stomach contents were identified in Atlantic sharpnose shark to complement stable isotope analyses and to help define dietary sources. The entire stomach, from the esophagus to the posterior portion of the scroll valve, was removed in the field and either frozen shipboard or placed on ice and frozen in a −20°C freezer upon return to the laboratory. In the lab, stomachs were thawed, opened, everted and rinsed over a 500 μm sieve. The bait used during the longline survey was Atlantic mackerel; therefore, all pieces of Atlantic mackerel found in stomachs were excluded from analysis. Stomach contents were identified to the lowest possible taxon, counted and weighed (0.1 g). Because of the overall low occurrence of identifiable gut contents in stomachs taken during the longline survey, additional stomachs were excised opportunistically from Atlantic sharpnose sharks caught during the NOAA MS Labs' annual longline surveys. For a detailed description of this survey, see Driggers et al. (2008). The presence of unidentifiable fish in the stomach contents, combined with the ecological similarity between certain prey types found in the stomachs (i.e. different species of squid) necessitated the pooling of stomach contents (Chipps and Garvey 2007). Stomach contents were pooled into four categories for analysis: crabs (portunid crabs), squid, shrimp (mostly penaeid shrimp) and fish (mostly unidentifiable).

The diet of the Atlantic sharpnose shark was assessed using four indices: percent by number (%N), weight (%W), frequency of occurrence (%O) (Hyslop 1980), and the index of relative importance (IRI, Piankas et al. 1971). To incorporate measures of variability, %N and % W were calculated for individual fish then summed for each prey type (Chipps and Garvey 2007), such that %N was calculated as the number of each prey type divided by the total number of prey types in that stomach. Similarly, %W was calculated as the weight of each prey type divided by the total weight of prey types from an individual stomach. %O was calculated as the number of stomachs containing a prey type divided by the total number of stomachs containing prey. The index of relative importance is a compound index that incorporates the previous three indices, expressed as IRI = %O (%N + %W). This product is then expressed as a percentage (%IRI) by dividing the total IRI for each prey type by the total IRI for all prey items (Cortés 1997). For the %IRI calculation, %N and %W were recalculated across all stomachs.

Statistical analyses

We employed univariate statistical tests to examine isotope and trawl data. To examine the effect of region (2 levels: eastern or western), season (6 levels: spring, summer or fall 2007 and 2008), size class (3 levels: young-of-the-year (YOY) 33–59 cm TL, juvenile, 60–84 cm TL and adult, >85 cm TL, Hoffmayer and Parsons 2003) and the ensuing interactions on stable isotope values for Atlantic sharpnose sharks, we used three-way, model I ANOVAs on C and N data for muscle and liver tissue. For brevity, results from these ANOVAs will be referred to by the combination of tissue (liver or muscle) and element (C or N) as follows: LC, LN, MC, and MN. Significant differences were further examined using a Tukey's post-hoc test with a sequential Bonferroni correction (Rice 1989). Two sample t-tests were used to test for a tissue effect on stable isotope values of C and N. To identify the presence of residual lipids in lipid-extracted shark liver tissue, Pearson's correlation was used to examine the relationship between C:N ratio and liver carbon isotope values. For fish and invertebrates caught in the ALMRD trawl during

2007 and 2008, we used a two-way, model I ANOVA to test for the effect of season (3 levels: spring, summer and fall) and region (western or eastern) on biomass. Unless otherwise noted, mean values are presented throughout with standard deviation in parentheses.

Results

Sample collection

Atlantic sharpnose sharks (Fig. 1b) were collected on bottom longlines during spring, summer and fall, throughout western (blocks 1 and 2) and eastern (blocks 3 and 4) regions. Eighteen cruises between March 2007 and November 2008 resulted in the capture of 376 Atlantic sharpose sharks, 212 of which were measured and released. Catch per unit effort for Atlantic sharpnose shark was greater in the western region (3.27 sharks/100 hooks/hour) compared to the eastern region (0.70 sharks/100 hooks/hour). One hundred sixty four Atlantic sharpnose sharks were retained for this study, most of which came from the western region, resulting in the analysis of 153 stomachs and 158 muscle and liver tissue samples (Table 1).

Analysis of log transformed ALMRD trawl biomass for fish and invertebrates revealed regional and seasonal differences in prey available to sharks throughout the year. For invertebrates, there was a significant interaction (F_2=3.36, p<0.05) between season and region such that biomass did not differ by season in the west, but in the east biomass peaked in the fall and was nearly absent in the spring and summer (Fig. 2a). Higher fish biomass was observed in the western region (Fig. 2b), although overall fish biomass did not differ significantly among season ($F_{5,26}$=1.55, p<0.21).

Stable isotope analysis

Stable isotope values indicated that Atlantic sharpnose sharks are tertiary consumers with basal C derived from a mixture of benthic and pelagic sources (Table 1, Fig. 3). N stable isotope values in Atlantic sharpnose sharks varied between tissues (t_{obs}=−4.29, df=317, p<0.01), but showed little within-tissue variation, averaging 14.14‰ (±0.79) in muscle and 14.49‰ (±0.64) in liver. These values translated to mean trophic position estimates between 4.4 (±0.36) and 4.6 (±0.29) calculated from muscle and liver tissues, respectively. A significant relationship was identified between C:N ratio and liver carbon isotope

Table 1 Mean values for $\delta^{13}C$ and $\delta^{15}N$ (± SD) and estimated trophic position (TP, ± SD) for Atlantic sharpnose shark. Factors correspond to either season (spring, summer or fall) or size class (young-of-the-year (YOY), juvenile or adult). Factors were analyzed independently to obtain sufficient sample size. No YOY samples were taken from the eastern region. n = number of samples analyzed

Region	Factor	n	δ13C (‰)		δ15N (‰)		TP		SD	
			L	M	L	M	L	M	L	M
West	Spring	37	−17.11 (0.49)	−16.91 (0.41)	14.58 (0.53)	14.20 (0.68)	4.6	4.4	0.24	0.31
	Summer	62	−16.78 (0.71)	−17.05 (0.38)	14.44 (0.60)	14.09 (0.84)	4.6	4.4	0.27	0.38
	Fall	44	−16.99 (0.72)	−17.10 (0.42)	14.54 (0.51)	14.31 (0.53)	4.6	4.5	0.23	0.24
	YOY	20	−17.25 (0.61)	−17.07 (0.47)	14.60 (0.56)	14.20 (0.68)	4.6	4.6	0.25	0.40
	Juvenille	73	−16.91 (0.71)	−17.04 (0.33)	14.36 (0.52)	14.09 (0.84)	4.5	4.4	0.24	0.33
	Adult	50	−16.83 (0.61)	−16.99 (0.48)	14.70 (0.56)	14.31 (0.53)	4.7	4.4	0.25	0.27
East	Spring	6	−17.17 (0.41)	−17.11 (0.37)	15.01 (0.48)	14.16 (0.94)	4.8	4.4	0.22	0.43
	Summer	7	−16.74 (0.70)	−16.70 (0.47)	13.88 (1.03)	13.74 (0.88)	4.3	4.2	0.47	0.40
	Fall	2	−16.14 (0.08)	−16.44 (0.10)	13.20 (1.28)	12.30 (1.91)	4.0	3.6	0.58	0.87
	Juvenille	10	−16.73 (0.69)	−16.80 (0.47)	14.00 (1.23)	13.52 (1.42)	4.4	4.1	0.56	0.21
	Adult	5	−17.04 (0.47)	−16.90 (0.42)	14.74 (0.54)	14.11 (0.64)	4.7	4.4	0.27	0.29

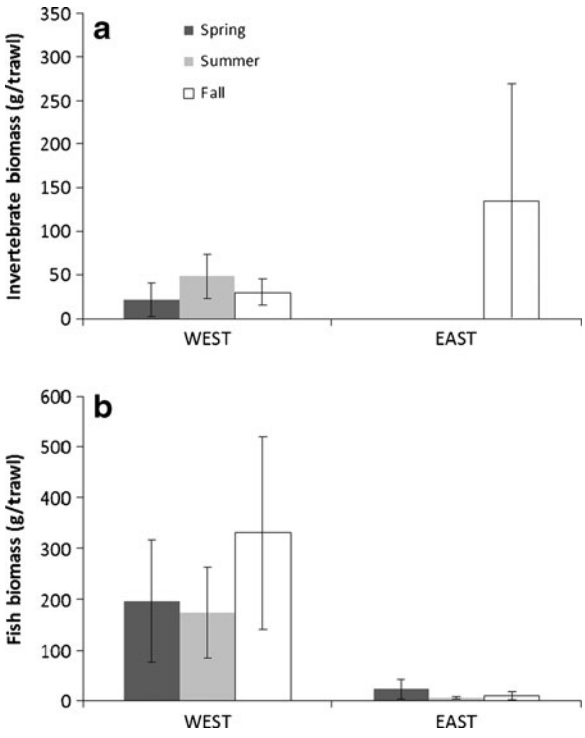

Fig. 2 Invertebrate (**a**) and fish (**b**) biomass shown by region and season. Error bars are SE. Data are taken from the ALMRD trawl survey, 2007–2008

values (r=0.91, p<0.01) suggesting incomplete removal of lipids during the lipid extraction process. Once lipid-normalized, carbon stable isotope values from liver tissue matched more closely to those from muscle tissue, although liver tissue was significantly more enriched on average (−16.92‰±0.68) than muscle (−17.01‰±0.42).

Stable isotope analysis of muscle tissue from potential prey items yielded a wide range of values for C and N (Table 2, Fig. 3). The mean $\delta^{13}C$ value was −19.64‰ (±1.19) for teleosts and −18.57‰ (±0.70) for invertebrates, suggesting that primary production for each of these groups is a mixture of benthic and pelagic carbon sources. The average $\delta^{15}N$ value was 13.13‰ (±0.33) for teleosts, higher and less variable than the mean $\delta^{15}N$ value for invertebrates (12.24‰±0.87). Stable isotope values from invertebrates seemed to have the most influence on the stable isotope composition of Atlantic sharpnose shark, in particular portunid crabs (*Callinectes* sp.), shrimp (*Penaeus* sp.) and mantis shrimp (Stomatopoda) (Fig. 3).

Nitrogen in liver tissue of Atlantic sharpnose sharks varied with size class (Tables 1 and 3). Nitrogen isotope values in liver tissue were similar between YOY and adult sharks, but depleted in juveniles (LN ANOVA, size class: F_2=4.277, p<0.02). Carbon isotope values in liver tissue did not vary as a function of size class, and there was no change with size class in muscle tissue for either isotope.

Stable isotope ratios from both muscle and liver tissue from Atlantic sharpnose sharks showed an interaction between region and season (Table 3, Fig. 4). Low seasonal variability was seen in the western region, with stable $\delta^{15}N$ and $\delta^{13}C$ values during spring, summer and fall. In contrast, higher variability has observed in the eastern region, which drove the regional x seasonal interaction. Eastern region $\delta^{15}N$ values in liver and muscle peaked in the spring, followed by steady decreases through the summer and fall (LN and MN ANOVA, region x season interaction, liver: F_4=3.60, p<0.01, muscle: F_4=4.84, p<0.01) (Fig. 4a and b). In contrast, carbon isotope values in liver (LC ANOVA, region x season interaction F_4=3.19, p<0.02) and muscle (MC ANOVA, region x season interaction F_4=3.72, p< 0.01) tissue from sharks in the eastern region were most enriched during summer and fall 2008 (Fig. 4c, d).

The relative contribution of prey items to the isotope signature in Atlantic sharpnose sharks varied spatially. In the western region, portunid crabs and shrimp were prominent in the diet of Atlantic sharpnose sharks, contributing approximately 50 and 25%, respectively, to the consumer's observed isotope signature (Fig. 5a). Mantis shrimp and squid (*Loligo* sp.) contributed approximately 10%, whereas the fish groups anchovy (*Anchoa* sp.), Atlantic croaker (*Micropogonias undulatus*), hardhead catfish (*Arius felis*) and searobin (*Prionotus* sp.) contributed little to nothing to the diet of Atlantic sharpnose sharks. More species contributed in greater proportions to the diet of Atlantic sharpnose sharks in the eastern region. Portunid crab had the largest relative contribution (~30%), whereas the other invertebrates contributed slightly more than the fish species (Fig. 5b).

Stomach content analysis

Stomach contents from 296 Atlantic sharpnose sharks (10% young of the year, 46% juvenile, and 44%

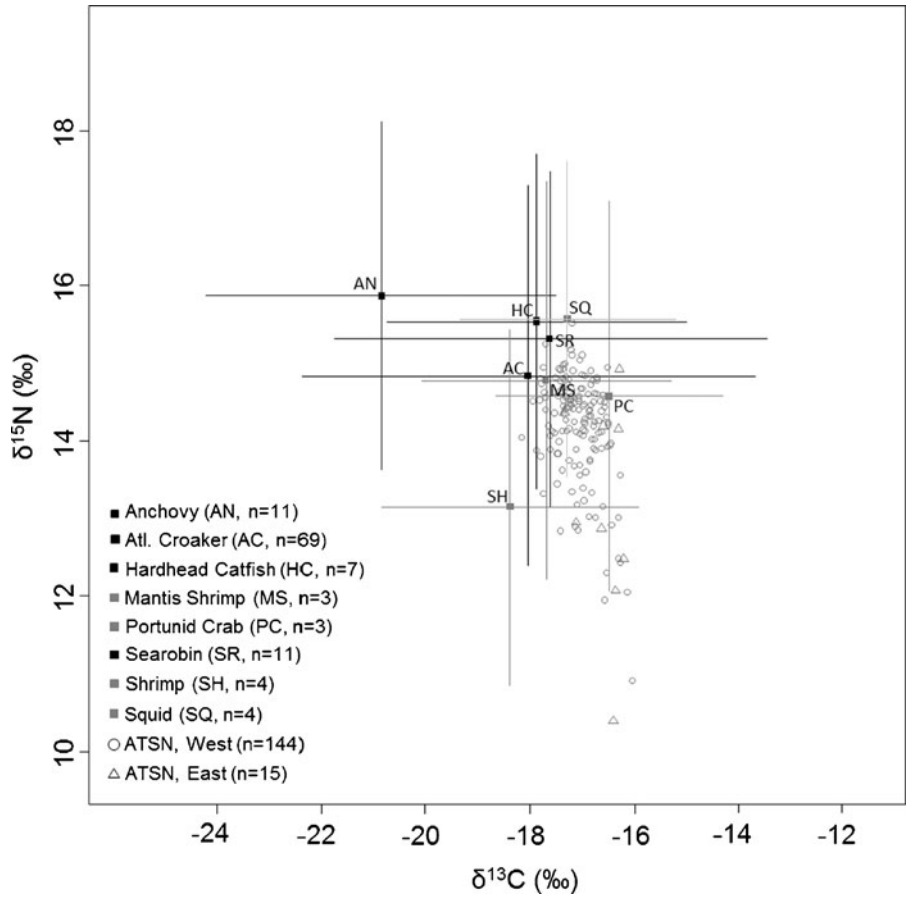

Fig. 3 Isotope biplot of individual $\delta^{15}N$ and $\delta^{13}C$ values of Atlantic sharpnose shark (ATSN) and mean $\delta^{15}N$ and $\delta^{13}C$ (± SD) of potential prey. All ATSN and potential prey are muscle tissue. Atlantic sharpnose sharks from the western region are shown with a circle, eastern region with a triangle. Fish are shown in black squares, invertebrates are shown in grey squares

adult) were examined. Thirty nine percent of recovered stomachs (n=115) were empty, and an additional eleven percent (n=34) contained only bait, leaving 147 stomachs (50%) for analysis. Fish, the majority of which were unidentifiable, comprised the majority of stomach contents. Diet varied little seasonally. % IRI values for fish were lowest in the summer (93.16%), coincident with the highest %IRI values for shrimp (3.93%) and crab (1.34%). Fish were even more prevalent in the diet of Atlantic sharpnose sharks sampled in the spring and fall, with %IRI values >98%. More variation in stomach contents was observed as a function of size class than season. Juveniles showed the broadest range of dietary items, although fish was still the most prevalent (IRI= 92.36%), followed by shrimp (%IRI=4.84). Our ability to examine regional differences in diet using stomach contents was hampered by low sample size in the eastern region (Table 4, Fig. 6).

Discussion

Mean trophic position and dietary sources

The mean trophic position range of 4.4 (±0.36) to 4.6 (±0.29) as estimated by muscle and liver tissue for Atlantic sharpnose sharks in this study is indicative of a piscivorous consumer and complements findings in previous studies. Atlantic sharpnose sharks have previously been reported to occupy a trophic level of 4.0 based on gut content analysis (Cortés 1999). Because stable isotopes detect assimilated diet, they account for metabolized prey that may be missed in stomach contents and would presumably give a more accurate account of trophic position than one calculated solely from gut contents. However, given the uncertainty of the parameters needed to calculate trophic level based on stable isotopes, such as variable nitrogen values at the base of the foodweb, unknown prey items and

Table 2 Mean values for $\delta^{13}C$ and $\delta^{15}N$ (± SD) for muscle tissue from potential prey items. n = number of samples analyzed

Potential prey: fish	n	δ13C (‰)	δ15N (‰)
Anchovy *(Anchoa* sp.)	11	−21.96 (1.36)	13.59 (0.51)
Atlantic croaker (*Micropogonias undulatus*)	69	−19.13 (1.05)	12.57 (0.71)
Hardhead catfish (*Arius felis*)	8	−18.96 (1.05)	13.26 (0.40)
Searobin *(Prionotus* sp.)	11	−18.70 (1.84)	13.04 (0.41)
Mean		−19.64 (1.19)	13.13 (0.33)
Potential prey: invertebrates			
Mantis shrimp (Stomatopoda)	3	−18.78 (0.67)	12.50 (0.80)
Portunid crab *(Callinectes* sp.)	3	−17.56 (0.43)	12.30 (0.77)
Shrimp *(Penaeus* sp.)	4	−19.47 (0.75)	10.87 (0.56)
Squid *(Loligo* sp.)	4	−18.37 (0.31)	13.29 (0.20)
Mean		−18.57 (0.70)	12.24 (0.87)

variable trophic fractionation factors, this is not necessarily the case. For instance, there are prey items previously identified as important in the diet of the Atlantic sharpnose sharks that fall outside the polygon of conceivable prey shown in Fig. 3 (e.g. anchovy). This suggests that either sharks are not in equilibrium with their diet, or that additional sources of prey not included in the isotope analysis are important to the diet of the Atlantic sharpnose shark.

Identifying dietary source as revealed by stomach content versus stable isotope analyses was made difficult by shortcomings with each method. The limited identifiable content in the stomachs of Atlantic sharpnose sharks suggested a diet composed primarily of fish, followed by squid and crustaceans,

Table 3 Summary table from four, three-way factorial ANOVAs examining the effect of size class (SC, fixed factor with 3 levels), region (R, fixed factor with 2 levels) and season (S, fixed factor with 6 levels) on stable isotope values of nitrogen and carbon in liver and muscle tissues for Atlantic sharpnose shark, 2007 and 2008. Bold values are statistically significant at α=0.05

Dependent Variable	R	S	SC	SC*R	SC*S	R*S	SC*R*S
Nitrogen							
Liver	0.22	0.06	**0.02**	0.24	0.42	**<0.01**	0.75
Muscle	0.12	0.83	0.82	0.90	**0.03**	**<0.01**	0.68
Carbon							
Liver	0.57	**0.02**	0.41	0.14	0.11	**<0.02**	0.97
Muscle	0.15	0.08	0.83	0.77	0.25	**<0.01**	0.91

findings in agreement with previous studies in this region that concluded Atlantic sharpnose sharks were generalists (Hoffmayer and Parsons 2003). Unfortunately, the most abundant fish sampled in the ALMRD trawl survey (Atlantic croaker, searobins and hardhead catfish) have similar isotope signatures and therefore provide little further resolution as to dietary source partitioning. Additionally, there may be nutritionally important prey items in the diet of Atlantic sharpnose sharks that were not captured by our sampling scheme. Previous studies have identified Gulf menhanden (*Brevoortia patronus*) as an important component in the diet of Atlantic sharpnose sharks (Barry 2002, Hoffmayer and Parsons 2003, Bethea et al. 2004, 2006). This fish is not sampled effectively by the gear used in the ALMRD trawl survey. Using stable isotope values for this fish collected in nearby waters ($\delta^{13}C$=−19.6, $\delta^{15}N$=11.9, Moncreiff and Sullivan 2001) and including those values in the mixing model, no additional dietary source resolution was gained because of the isotopic similarity of Gulf menhaden to other potential prey in our model. Future efforts to identify dietary sources in this species using isotope mixing models will require more identifiable stomach contents to direct the choice of prey for isotope sampling.

Regional and seasonal differences in diet

Atlantic sharpnose shark liver and muscle tissue showed regional and seasonal differences in stable isotope values. The fact that both tissues demonstrate this effect suggests the sampled sharks are following consistent dietary patterns. Liver and muscle tissue

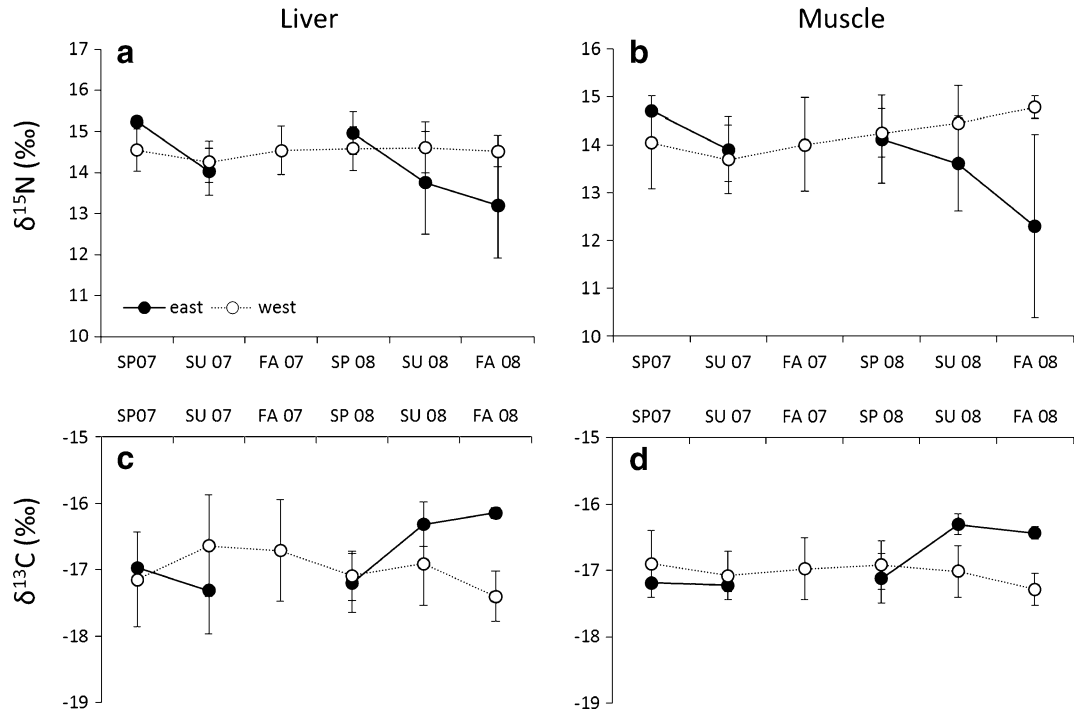

Fig. 4 Tissue-specific mean values for (**a**) $\delta^{15}N$ in liver, (**b**) $\delta^{15}N$ in muscle, (**c**) $\delta^{13}C$ in liver and **d**) $\delta^{13}C$ in muscle, (± SE) for Atlantic sharpnose sharks. Some SE are too small to be visible. No data were collected in the eastern region during fall 2007

are known to have different turnover rates with liver turnover on the order of months compared to muscle turnover on the order of years (MacNeil et al. 2006; Logan and Lutcavage 2010). However, many studies quantifying tissue turnover in elasmobranchs have used relatively slow-growing species (e.g. Logan and Lutcavage 2010), such as the sandbar shark (Sminkey and Musick 1995). Conversely, the Atlantic sharpnose shark is relatively fast growing (Loefer and Sedberry 2003), which may result in faster tissue turnover in this species and hence may record shifts more acutely. Using stable isotope values from prey items known to be part of the diet of Atlantic sharpnose shark in the northern Gulf of Mexico (based on diet information in Hoffmayer and Parsons 2003), Atlantic sharpnose shark collected in the western region of our study had a high a proportion of invertebrates in their diet (shrimp and crabs). This pattern was relatively consistent over the study leading to relatively stable $\delta^{15}N$ values. In contrast, Atlantic sharpnose shark collected from the eastern region showed higher variability in prey, which was reflected in the more variable $\delta^{15}N$ values. If we assume that the isotope signature of Atlantic sharpnose sharks sampled in the western region represent the general condition of that species in that region, then our data suggest plasticity in feeding across a relatively small spatial scale.

The relatively low mean and high variance associated with prey contributions in the eastern region are in contrast to the western region, but are supported by trawl data that demonstrate large differences in available fish and invertebrate biomass between regions. While Atlantic sharpnose sharks undoubtedly move across the eastern and western regions in this study, acoustic telemetry studies have shown mean daily 50% kernel home ranges for this species to be relatively small (1.64 km^2; Carlson et al. 2008). Additionally, this species is thought to display multiple forms of residency, including some degree of philopatry (Carlson et al. 2008). We suggest that the sharks sampled in this study reflect the average condition of the population from the region or season they were sampled in, although additional telemetry studies and higher sample sizes from the eastern region are necessary to confirm this.

Although we conclude that the most parsimonious explanation for the variable $\delta^{15}N$ values in the east is

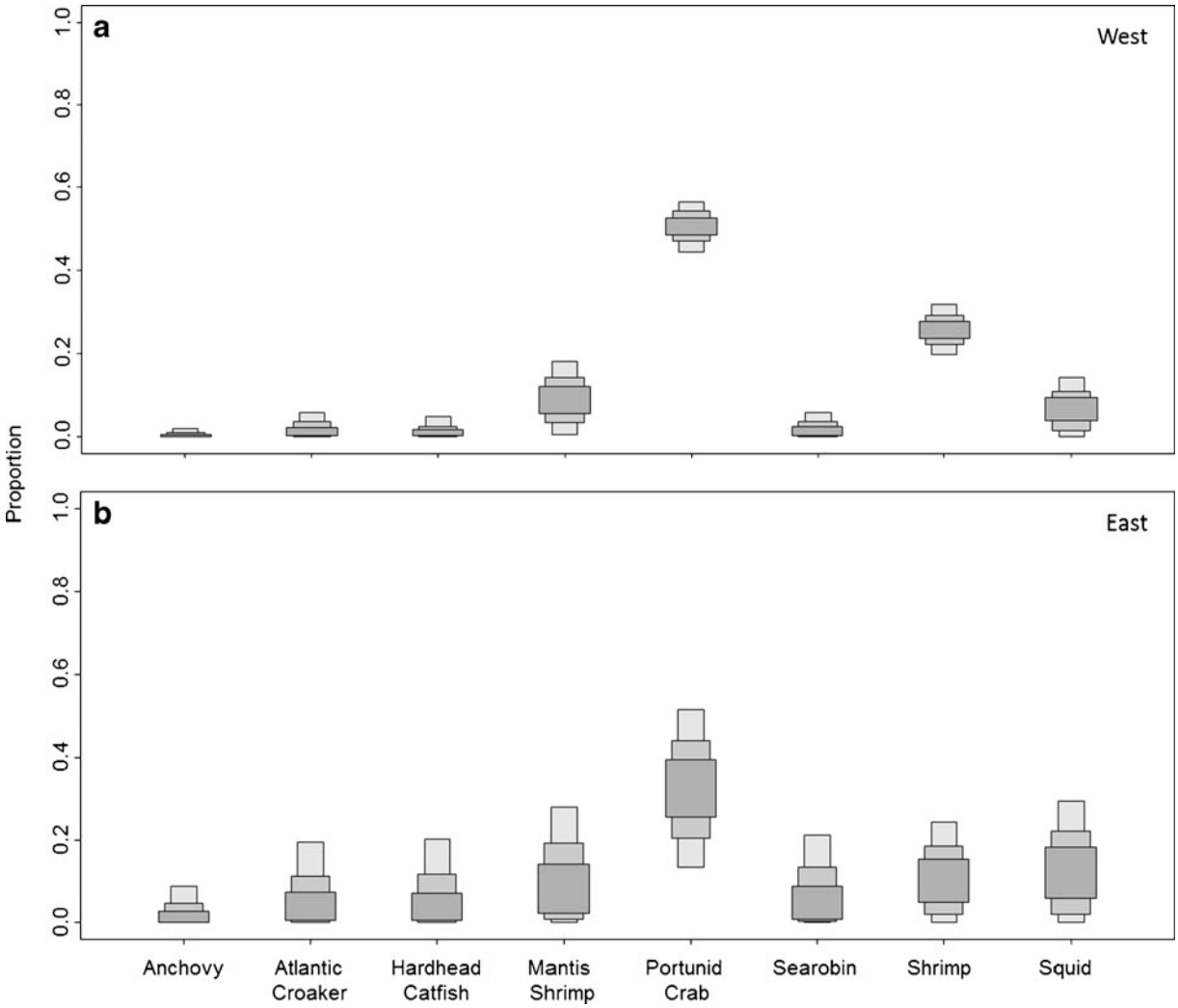

Fig. 5 Boxplots showing the relative contributions from potential prey sources to the diet of Atlantic sharpnose shark sampled (**a**) west and (**b**) east of Mobile Bay using SIAR. The proportions show the credibility intervals at 95, 75 and 25%

a plastic diet in this region owing to shifts in the available forage base (supported by high variability in our trawl data in the east), seasonal variability in $\delta^{15}N$ values may occur as a result of changes in the source N pool or movement of Atlantic sharpnose shark outside the region of our study (i.e., further offshore in the winter). N_2 fixation by *Trichiodesmium* has been shown to be an important component of primary production in the offshore waters of the Gulf of Mexico (Holl et al. 2007). In this case, we would expect baseline $\delta^{15}N$ values (and hence, Atlantic sharpnose shark $\delta^{15}N$ values) to be lowest in the spring. This influx of organic matter in the spring could lower values throughout the foodweb; however, this high N_2 fixation would need to disproportionately affect Atlantic sharpnose sharks collected in the eastern region. Another potential complication in the interpretation of our $\delta^{15}N$ values is the likely winter movement of Atlantic sharpnose sharks to offshore waters. While in offshore waters Atlantic sharpnose sharks could switch to higher trophic level prey as has been shown in other Gulf of Mexico predators (Barros et al. 2009).The most enriched $\delta^{15}N$ values in our study were seen in the spring; given the relatively slow turnover of muscle tissue, these values may reflect periods of offshore feeding during the winter, either via feeding on different trophic level prey, or by feeding in a system with different baseline isotope

Table 4 Percent by number (%N), percent by weight (%W), percent relative occurrence (%O) and the index of relative importance, expressed as a percent (%IRI) as a function of season (spring, summer, fall), size class (YOY, juvenile, adult), and region (west, east). %N and %W values were calculated on individual stomachs (mean values ± SD). For %IRI calculations, %N and %W values were re-calculated across all stomachs. *n* = sample size–no sample size is given for the compound index %IRI

Index	Factor	Level	n	Prey category			
				Crab	Fish	Shrimp	Squid
%N	Season	Spring	24	0.00	85.42 (0.31)	12.50 (0.30)	2.08 (0.10)
		Summer	60	8.33 (0.24)	68.75 (0.44)	16.25 (0.36)	6.67 (0.23)
		Fall	47	2.83 (0.15)	84.75 (0.32)	9.22 (0.26)	3.19 (0.16)
		YOY	11	0.00	90.91 (0.29)	9.09 (0.29)	0.00
	Size class	Juvenile	63	7.67 (0.24)	68.92 (0.43)	17.06 (0.36)	6.35 (0.23)
		Adult	61	2.46 (0.14)	85.52 (0.32)	8.74 (0.26)	3.28 (0.15)
	Region	West	68	1.41 (0.08)	81.86 (0.35)	11.52 (0.30)	5.15 (0.19)
		East	7	0.00	100.00 (0)	0.00	0.00
%W	Season	Spring	19	0.00	88.23 (0.30)	7.90 (0.24)	0.00
		Summer	55	8.73 (0.26)	65.79 (0.46)	7.21 (0.19)	11.43 (0.30)
		Fall	44	3.03 (0.16)	85.60 (0.33)	6.07 (0.19)	2.27 (0.15)
		YOY	9	0.00	88.89 (0.31)	5.56 (0.16)	0.00
	Size class	Juvenile	57	8.89 (0.26)	65.23 (0.45)	8.42 (0.22)	11.91 (0.31)
		Adult	56	1.90 (0.13)	88.27 (0.31)	5.07 (0.18)	0.89 (0.07)
	Region	West	57	1.40 (0.10)	81.92 (0.38)	5.74 (0.23)	10.53 (0.31)
		East	5	0.00	100.00 (0)	0.00	0.00
%O	Season	Spring	19	0.00	13.82	3.25	0.81
		Summer	55	5.69	31.71	8.94	4.07
		Fall	44	1.63	31.71	4.88	1.63
		YOY	9	0.00	6.51	0.81	0.00
	Size class	Juvenile	57	5.69	33.30	10.57	4.07
		Adult	56	1.62	40.65	5.69	2.44
	Region	West	57	1.62	39.02	8.13	4.07
		East	5	0.00	4.07	0.00	0.00
%IRI	Season	Spring	–	0.00	98.02	1.86	0.12
		Summer	–	1.34	93.16	3.93	1.57
		Fall	–	0.02	98.39	1.37	0.22
		YOY	–	0.00	98.61	1.39	0.00
	Size class	Juvenile	–	1.22	92.36	4.84	1.59
		Adult	–	0.07	98.93	0.86	0.14
	Region	West	–	0.07	97.27	1.90	0.77
		East	–	0.00	100.00	0.00	0.00

values. Although the current study lacks the temporal resolution to fully resolve these alternative explanations, neither provides a strong mechanistic explanation to account for the disparity between eastern and western regions.

Our assessment of regional differences in feeding for the Atlantic sharpnose shark was impeded by the lower sample size for sharks collected in the eastern region. Although not ideal, our sample size (n=15) from this area is comparable to other studies. While the lower sample size in the eastern region may naturally lead to higher variability, the variation in Atlantic sharpnose shark stable isotope data was also observed in the trawl data, for which sample size was more balanced between regions. Moreover, the disparity in sample size between the eastern and western regions directly reflected the distribution of these sharks as shown by our catch data. Future studies addressing dietary habits of Atlantic sharpnose shark in this region will benefit from increased sample size in the area east of Mobile Bay.

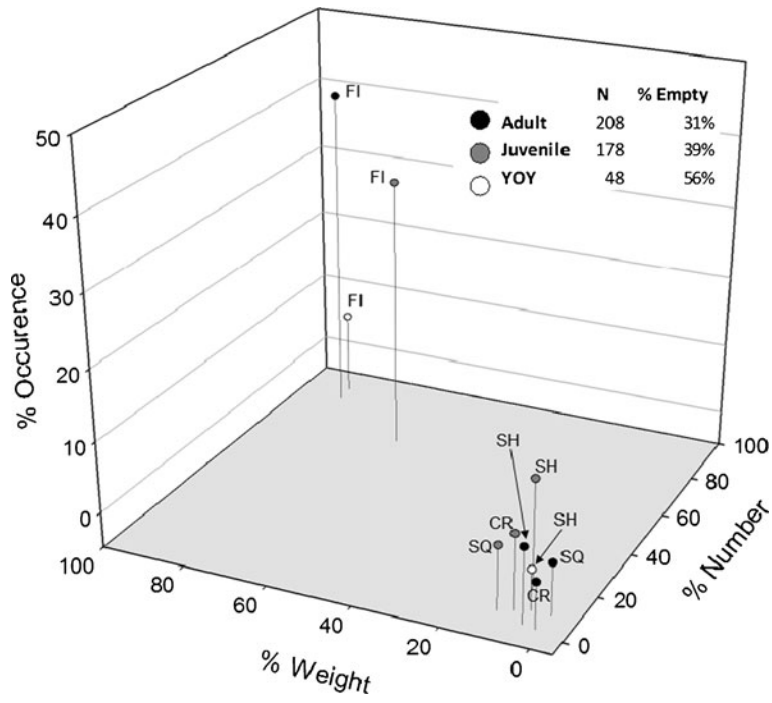

Fig. 6 Stomach content data as a function of size class. N is equal to the number of stomachs obtained. FI = Fish, CR = Crabs, SH = Shrimp, SQ = Squid

Ontogenetic shifts

Stomach content as well as carbon and nitrogen stable isotope data from liver tissue highlighted a dietary shift with size class. Such shifts have been previously documented for this species in the Gulf of Mexico using stomach content analysis (Hoffmayer and Parsons 2003; Bethea et al. 2006). Our findings support this body of work, and provide interesting insight from stable isotope analysis. For both carbon and nitrogen, YOY and adult signatures were similar, with contrasting signatures in juveniles. The nitrogen and carbon isotope signatures of embryos in this species (McMeans et al. 2009) as well as other placentatrophic sharks (Vaudo et al. 2010) have been shown to be elevated compared to the mothers. Our data further support this, and suggest that postpartum, the isotope signatures of YOY sharks are lower than during gestation, i.e. intermediate between the relatively enriched state in-utero and the relatively depleted state as juveniles feeding on lower trophic level prey.

Our study is the first to use stable isotope ratios of C and N, as well as stomach content analysis, to examine the feeding ecology of the Atlantic sharpnose shark in the GOM. Isotopic assignment of trophic position was within the range of estimates previously suggested based on gut contents alone. Stomach content analysis was hampered by a lack of identifiable prey items, whereas identifying dietary source with stable isotopes was difficult because of the similarity in isotopic signatures among potential prey items. Despite the individual limitations, both techniques in tandem supported the same conclusions and thereby strengthen our findings. The seasonal and regional differences identified in muscle and liver tissue suggest that sampled sharpnose sharks are following relatively consistent dietary patterns which appear more plastic in the eastern region compared to the western region, perhaps as a result of regional differences in forage base. Both stomach content and stable isotope analyses suggest a shift in diet with size class, in agreement with previous studies. Future investigations should consider the use of the stable sulfur isotope, which may further resolve basal C sources. Additionally, the use of compound-specific analyses would negate the need to isotopically identify the base of the foodweb, therefore providing a more precise estimate of trophic position. Our findings suggest small scale shifts in trophic position and carbon source may need to be considered when constructing coastal foodweb models that incorporate elasmobranchs.

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